APPENDIX A: TROUBLE SHOOTING

1 GENERAL BIOMEK® 2000 AUTOMATION WORKSTATION

1.1 BioMek® 2000 Base Unit

An emergency stop button is located at the lower front area on the robot, just below the deck. If this button is pressed, the robot will abort the method it is currently running. The emergency stop only needs to be used when the robot could be damaged by crashing into the deck or a tool could be damaged by crashing into something on the deck. If what is desired is simply to stop the method while it is running, simply click on the "Stop" button. The method will then pause and several options will be available. The button labeled "Continue" can be clicked to resume the method, the button labeled "Trace" can be clicked to advance to the next step (this function is useful when calibrating), the button labeled "Go Up" will move the pod up and the button labeled "Quit" will terminate the method.

1.2 Shaker

If the computer is shut down for any reason, once it has been re-booted, the shaker must be turned off, then back on in order to reset and put back on line with the computer. The communication between the computer and the shaker must be restored. This will occur once a method is started. The shaker is very susceptible to minor power perturbations that can throw it off line with the computer. If there is any reason to suspect that the power may have fluctuated, the shaker should be turned off then turned back on to reset and place on line with the computer. If the method is already running and the shaker fails to respond when directed, an error box will come up on the computer screen. When the shaker window comes up and the shaker fails to respond, manually click on shaker and it will shake at the programmed settings. After that the shaker will chirp and the LED display will show an abnormal readout. Allow the shaker to chirp until the next shaking step. It will ordinarily respond to the command and be placed back on-line. If it fails to do so, it may be necessary to turn the shaker off for 1 minute. Then turn the shaker back on to reset. The Shaker may have to initially be manually commanded to shake from the computer screen window. If all previous steps fail, call Technical Support at Promega Corporation.

1.3 Pipet Tools

The seal between the pipette tool and the corresponding tips may not be perfect and this can result in inconsistent pipetting, which is most noticeable with the multi-channel pipette tool. Often, the pipettor probes simply need to be wiped off with a KimWipe and 95% Ethanol or Isopropyl alcohol. If no improvement in the pipetting consistency is observed, then the pipette tool may need to be serviced as this can be an indication that the quad rings need to be changed.

2 AluQuant® Human Quantitation System/AluQuant® Calculator v3.0

2.1 Deleting bad data points from the Standard curve.

A minimum of 5 data points, including the water blank, are necessary for the standard curve. Occasionally, data points from the standard need to be deleted to reduce elevated background

and/or to produce a more linear standard curve. The data points may be deleted, then the other standards and their RLU values must be copied and pasted into the cells so that the standard table is continuous. See Figures 1A and 1 B for an example. The Calculate button must be pressed again in order to generate a new extrapolated curve and to correct the DNA sample concentrations that may be off.

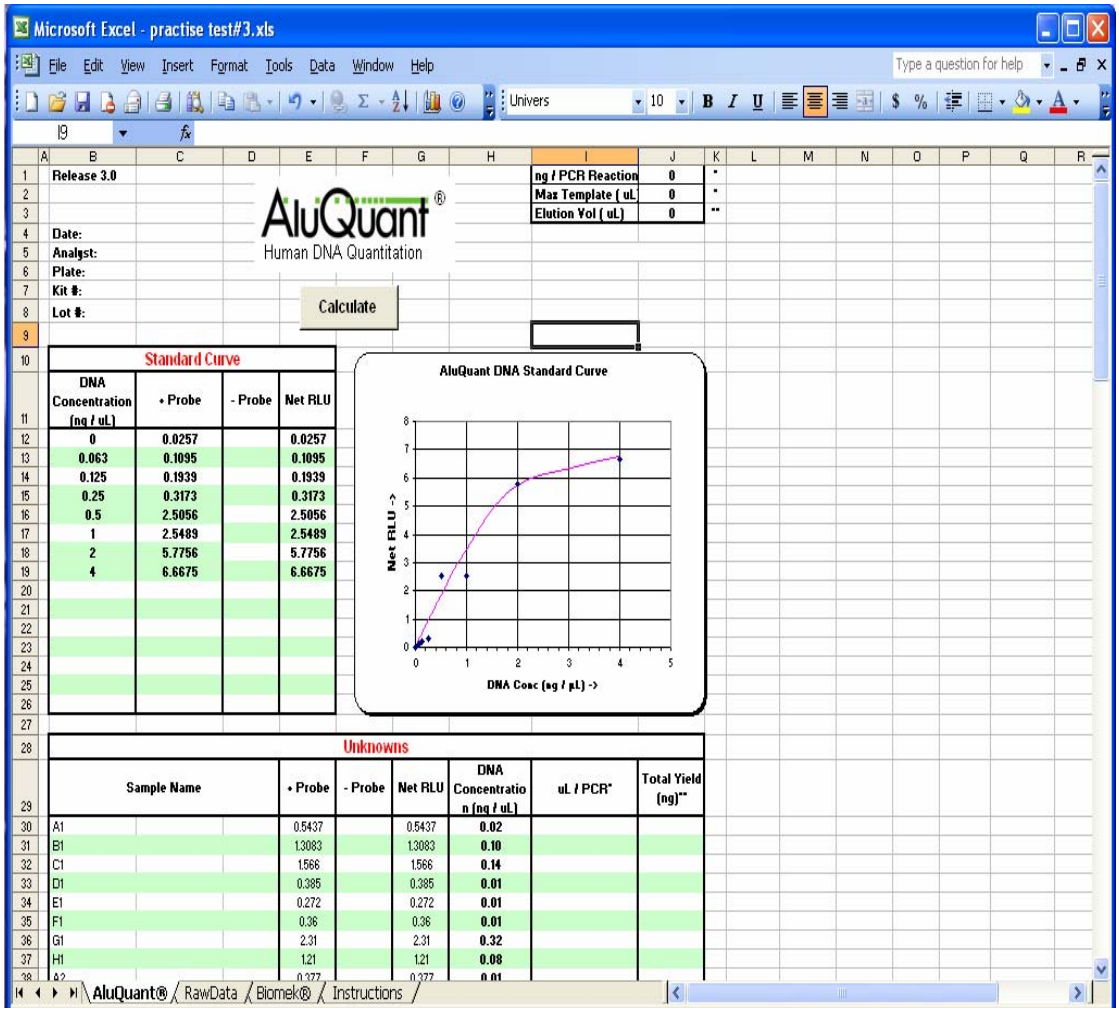

Figure 1A. Standard curve which may need data points deleted

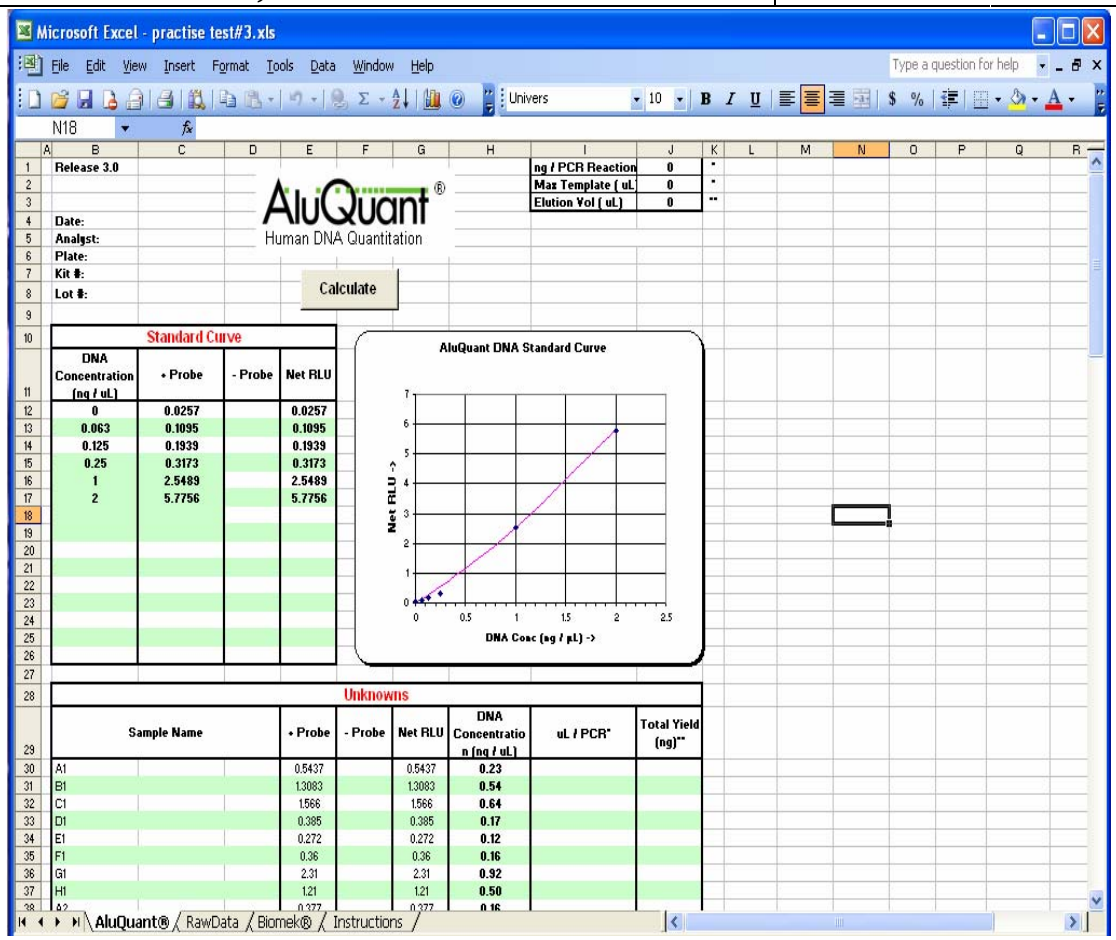

Figure 1B. Deletion of data points from standard curve

NOTE: Figures 1A and 1B illustrate the large difference in estimated DNA concentration for the samples when data points are deleted from the standard curve. Although AluQuant® Calculator v3.0 doesn't flag the user as to irregularities with the standard curve because the quadratic formula is capable of fitting any curve, it is imperative that the user evaluate the slope and character of the extrapolated (pink) curve to ensure the most accurate DNA concentration estimation. A large dip or even a flattening out in the RLU value for the 4 ng/µL standard will usually require that it be deleted since it can throw off the estimates for the most concentrated samples. The desired slope for the standard curve is approximately 45°.

2.2 Extremely low RLU values

It is normal for the RLU values to vary somewhat from run to run and they will usually halve when the L/L reagent is used that has been reconstituted and frozen. If the RLU values are not greater than 1.0-2.0 RLUs for the 4 ng/µL standard, even if the standards reduce in value proportionately with the lower concentrations as shown in Figure 2, it is likely that the luminometer lens is obscured. This can occur when the injector tip becomes clogged and mis-injects into the luminometer plate, hitting the top of the plate instead of into the wells. Another symptom of an obscured lens is the occasional large over estimate of a sample. This is due to the fact that the lens is usually not uniformly obscured so that most of the signal may

be muted, but an occasional sample signal may be detected at full strength and is thus vastly out of proportion with the muted standards.

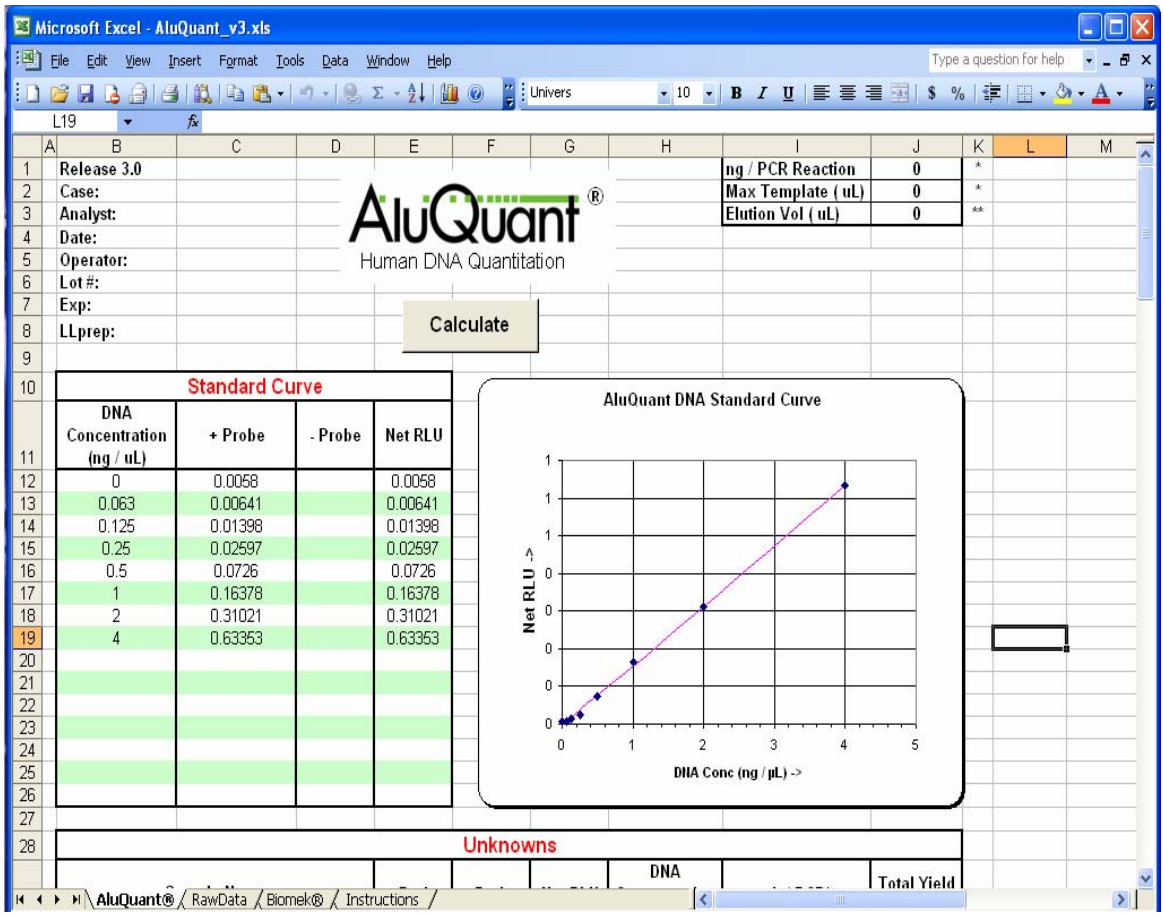

Figure 2. Abnormally low RLU values.

2.2.1 If extremely low RLU values are observed, the lens must be cleaned as described in Appendix E.

2.3 Inconsistent quantitation data.

2.3.1 Quantitation will become inconsistent if blockage of the injection tip occurs. Sometimes the data will become inconsistent before it is apparent that the injector tip is blocked. If inconsistent data is observed, then change the injection tip and test the instrument to determine if consistency has improved.

2.3.2 Quantitation will become inconsistent if the injector tubing becomes kinked. If replacing the injector tip fails to restore consistency to the quantitation data, then the injector tubing should be checked. The tubing is thin and can be easily bent, which can possibly impede the injection of the full 50 µL aliquot of L/L reagent. New

tubing should be used to replace the injector tubing by following the directions in the luminometer manual for replacing the injector tubing.

2.3.3 The Teflon seal of the plunger can also fail over time. Inconsistent data can also be a symptom that the plunger needs replacement. The plunger generally should be replaced every 3-5 years depending on usage. If replacing the tip and the tubing fails to rectify the issues with inconsistency, then the plunger may need replacement.

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